

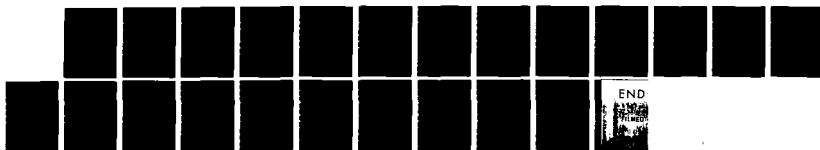
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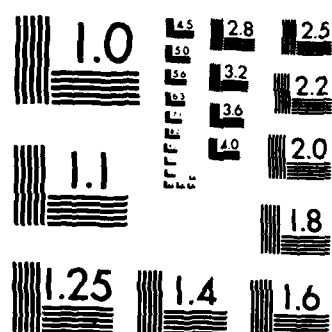
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AN INVESTIGATION OF MEMBRANE-ENCAPSULATED TRYPANOCIDES

ANNUAL REPORT

KARL J. HWANG

JANUARY 15, 1979

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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as vehicles to prolong an encapsulated drug in circulation for extended periods of time. The routes of the administration of this type of liposomes do not seem to alter the blood clearance time of the small unilamellar liposomes. However, the extent of hepatic uptake of liposomes seem to decrease, if liposomes are given by intraperitoneal injection instead of intravenous injection. Similar results have also been observed in liposomes of about 650Å in diameter, except that an extensive uptake of liposomes by skin has been found within 2 hrs. after the intraperitoneal injection of liposomes. This suggests that the absorption of liposomes may be via skin, if liposomes are injected into the peritoneal cavity. Furthermore, the complex of ethylene-diaminetetraacetic and $^{111}\text{In}^{3+}$ may be a better marker than $^{99\text{m}}\text{TcO}_4^-$ in future studies of lipid-protein vesicles of serum.

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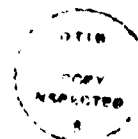
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Summary

This report describes the progress of research in two areas, namely, the degradation of liposomes in tissues and the prolongation of drug carriers in blood circulation. In the area of the degradation of liposomes, a general method of loading $^{111}\text{In}^{3+}$ to liposomes by a mobile ionophore, 8-hydroxyquinoline, has been developed to encapsulate a high specific radioactivity of $^{111}\text{In}^{3+}$ in liposomes for investigating the fate of liposomes in tissues. Liposomes prepared by 8-hydroxyquinoline ($^{111}\text{In}^{3+}$) loading procedure have a similar tissue distribution as liposomes prepared by standard methods of encapsulation. Furthermore, the result of the encapsulation of unilamellar liposomes by multilamellar liposomes has indicated that there is a great potentiality in utilizing a special type of liposomes, in which only the central cores of each multilamellar liposomes contain $^{111}\text{In}^{3+}$ for elucidating the modes of the degradation of liposomes in tissues.

In the study of the prolongation of drug carriers in blood circulation, our results suggest that unilamellar liposomes of about 350Å in diameter can serve as vehicles to prolong an encapsulated drug in circulation for extended periods of time. The routes of the administration of this type of liposomes do not seem to alter the blood clearance time of the small unilamellar liposomes. However, the extent of hepatic uptake of liposomes seem to decrease, if liposomes are given by intraperitoneal injection instead of intravenous injection. Similar results have also been observed in liposomes of about 650 Å in diameter, except that an extensive uptake of liposomes by skin has been found within 2 hrs. after the intraperitoneal injection of liposomes. This suggests that the absorption of liposomes may be via skin, if liposomes are injected into the peritoneal cavity. Furthermore, the complex of ethylene diaminetetraacetic and $^{111}\text{In}^{3+}$ may be a better marker than $^{99\text{m}}\text{TcO}_4^-$ in future studies of lipid-protein vesicles of serum.

Forward

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences - National Research Council.

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I. Degradation of Liposomes in Tissues

1. In Vitro Study

A. The Development and Application of Techniques for Preparing Liposomes Encapsulating a High Specific Radioactivity of ^{111}In .

The objective of this study is to develop a general method to encapsulate a high activity of $^{111}\text{In}^{3+}$ for studying the fate of liposomes in tissues. In some of the study, such as the preparation multilamellar liposomes in which only the central core of the unilamellar liposome contains $^{111}\text{In}^{3+}$, the use of unilamellar liposomes with a high specific activity of $^{111}\text{In}^{3+}$ is required. Furthermore, if the 2% yield of encapsulation of $^{111}\text{In}^{3+}$ by the current standard methods can be improved, it will not only mean a saving of materials but also a saving of our time and effort in preparing enough liposomes for various studies.

The strategy adopted for increasing the yield of encapsulation of $^{111}\text{In}^{3+}$ was to utilize a lipid soluble ionophore to carry $^{111}\text{In}^{3+}$ across the lipid bilayer of liposomes which encapsulate a chelating agent serving as a sink to trap the $^{111}\text{In}^{3+}$. 8-Hydroxyquinoline (8-HOQ) was chosen as the ionophore of $^{111}\text{In}^{3+}$ and 1 mM nitrilotriacetic acid was encapsulated in liposomes as the trapping agent.

Previously, we have been able to load $^{111}\text{In}^{3+}$ ions to unilamellar liposomes of neutral surface charge, such as L- α -dipalmitoyl phosphatidylcholine (DPPC) liposomes or DPPC: cholesterol (2/1; M/M) liposomes, with an entrapping efficiency up to 99%~100% (1). Attempt to apply this technique for loading In-111 ions to liposomes of various lipid compositions has been tried. The results of the studies can be summarized as follows:

(i) The loading efficiency of $^{111}\text{In}^{3+}$ to liposomes is found to be pH dependent. The loading efficiency is usually low, when the pH in the interior of the liposome is below 5.5. An isotonic buffer solution of 0.106M sodium phosphate, pH 7.4 was thus chosen for all subsequent studies.

(ii) The loading efficiency depends on the partition coefficient of 8-HOQ($^{111}\text{In}^{3+}$) complex in lipid and aqueous phases. Therefore, instead of a 99%-100% of entrapping efficiency an encapsulating efficiency of 70% is usually the case. This is still far better than an entrapping efficiency of 2%-3% by the current standard methods of encapsulation.

(iii) A simple method of removing the untrapped $^{111}\text{In}^{3+}$ or 8-HOQ($^{111}\text{In}^{3+}$) which is still embedded in the lipid bilayer of liposomes has been developed. This involves the use of diethylenetriaminepentaacetic acid (DTPA) to chelate $^{111}\text{In}^{3+}$ in the exterior of liposomes, which can be separated from the DTPA ($^{111}\text{In}^{3+}$) complexes by a subsequent passage of the liposome suspension through a microcolumn of an anion exchange resin (AG1-X3 in phosphate form). Almost all DTPA ($^{111}\text{In}^{3+}$) complexes are adsorbed to the column. About 90% of the liposomes can be recovered. Since this method has a minimal dilution effect, liposomes can be prepared by repeat chromatographic purification. We found that the removal of 8-HOQ ($^{111}\text{In}^{3+}$) embedded in the lipid bilayer of liposomes sometimes required a repeat chromatographic purification.

(iv) The leakage of liposomal contents from positive unilamellar liposomes of DPPC: cholesterol: stearylamine (62:30:8 in molar ratio) and negative unilamellar liposomes of DPPC: cholesterol: dicetylphosphate (62:30:8 in molar ratio) appeared to be slightly more than that of neutral liposomes of DPPC: cholesterol (2:1 in molar ratio). Work is in progress to investigate whether or not such a leakage is the intrinsic properties of these charged liposomes, which contain detergent like single chain molecules, rather than due to the method of loading itself. A comparison of the results from standard encapsulation of $^{111}\text{In}^{3+}$ in liposomes with our new method of loading $^{111}\text{In}^{3+}$ to liposomes will resolve this problem.

B. Encapsulation of Unilamellar liposomes by Multilamellar liposomes

The preparation of a special type of multilamellar liposomes, in which only the central core and not the outer shells of each onion-like spherules entraps $^{111}\text{In}^{3+}$, will help us elucidate the mode of degradation as well as the kinetics of degradation of liposomes in tissues. Furthermore, one may also use this type of liposomes to investigate whether or not it is possible to modulate the rate of degradation of liposomes in tissue by encapsulating inhibitors of phospholipases, such as a strong chelating agent, in the outer shells or by encapsulating agents which might enhance the liposome-phagosome fusion process in the outer shells of a multilamellar liposome. Research along this line will not only increase our basic knowledge in terms of the modes and the kinetics of the degradation of liposomes, but also provide us very valuable information concerning the design of a drug carrier for various schemes of treatment, such as the reduction of the possible toxic effect of liposomes to the host by enhancing the rate of liposome-degradation or the prolongation of the sustained release of trypanocides by reducing the rate of the degradation of liposomes in tissues, etc.

The approach of preparing this type of liposomes was derived from the observation that if dry phosphatidylcholine is suspended in an aqueous solution by vortex or by a brief sonification at low energy, the majority of the liposomes in the resulting suspension are multilamellar. This suggests that under normal condition a smaller liposome tend to be encapsulated by a larger liposome. To test this idea of liposomes encapsulating liposomes, a suspension of DPPC unilamellar liposome entrapping some $^{111}\text{In}^{3+}$ was used to suspend soybean-phospholipids by a brief sonification in a water bath. Using the technique of perturbed angular correlation (PAC) to monitor the leakage of ^{111}In we found that the serum-induced leakage of $^{111}\text{In}^{3+}$ from DPPC liposomes was reduced to a negligible level after the sonification. This implied soybean-phospholipids can encapsulate DPPC unilamellar to form multilamellar liposomes, masking the perturbing effect of serum proteins to DPPC liposomes. Furthermore, the yield of this type of encapsulation must have been very high as indicated by the small amount of leakage of $^{111}\text{In}^{3+}$ from DPPC liposomes after the addition of serum to the resulting liposome suspension.

Efforts have been directed to investigate whether or not this phenomenon of liposomes encapsulating liposomes is a general property of liposomes and how other factors, such as the composition of lipids, the physical and chemical properties of lipids, the amount of lipids used, and the time of sonification can affect the yield of encapsulation. The criterion for estimating the yield of encapsulation was based on either the percentage of radioactivity of

¹¹¹In in unilamellar liposomes which can be spun down as a pellet at 1000 g for 20 min. after the process of encapsulation by bath sonification or the percentage of the radioactivity observed in the void volume of a Sepharose 4B column. A preliminary result of our study can be summarized as follows:

(i) The method of separating multilamellar liposomes from unilamellar liposomes by centrifugation is a very convenient method. However, not all multilamellar liposomes can be spun down at 1000g for 20 min. This is especially true for liposomes with lipids of low liquid-crystalline melting temperature and liposomes with either positive or negative surface charge. In the encapsulation of DPPC unilamellar liposomes by using three times of the amount of DPPC: cholesterol (2:1 in molar ratio) lipid mixture, the yields of encapsulation were 12%, 27%, 42% and 62%, when the times of sonification were 5 min., 10 min., 15 min., and 35 min., respectively. The sonification temperature was performed at 52°C which is about 10°C above the phase transition temperature of DPPC.

(ii) The method of separating multilamellar liposomes from unilamellar liposomes by gel filtration chromatography in Sepharose 4B appeared to be a more practical approach, even though the adsorption of liposomes by the column may introduce some errors in our estimation of the yield of encapsulation. In a similar condition as described in (i), the yield of encapsulation of DPPC unilamellar liposomes by three times of the amount of DPPC: cholesterol: sterylamine (62:30:8 in molar ratio) lipid mixture was 82%, when the sonification-time was 25 minutes.

Our result is too preliminary to come to a conclusion concerning the effects of various factors on the yield of encapsulation. However, the above results demonstrate that the observed high yield of encapsulation will allow us to prepare this type of liposomes encapsulating enough ¹¹¹In for future study of the degradation of liposomes in tissues.

2. In Vivo Study

A. An Estimation of the Contribution of Blood-Radioactivity in Each Organ of a Mouse.

In the investigation of the tissue distribution of a drug carrier by radioactive-tracer techniques, the radioactivity observed in an organ is resulted from two main sources, namely, the radioactivities from blood and the organ. In order to know the true amount of drug carrier taken up by an organ, the percentage of the total blood perfused in the organ should be determined.

To do this, we utilized 8-HOQ(¹¹¹In⁺³) labeled-erythrocytes of a mouse to estimate the blood volume in each organ. The procedure of labeling mouse erythrocytes by 8-HOQ(¹¹¹In³⁺) was a slight modification of the method of Welch (2). Briefly, fresh mouse blood was isolated from a mouse using 3.8% sodium citrate as an anticoagulant. Erythrocytes were washed three times by phosphate buffer saline, pH 7.4 (PBS) before incubating with 10μCi of 8-HOQ(¹¹¹In³⁺) at room temperature for 30 min. Care was taken to remove the buffy coat during the washing process. The labeling reaction was stopped by washing the cells with PBS twice and once by the citrated mouse plasma. Labeled erythrocytes were finally suspended in citrated mouse plasma for transfusing to six recipients. The average numbers of the percentage of blood volume of each organ from these six mice is shown in Table 1.

The data of blood volumes were used as an input data for calculating the tissue distribution of liposomes in mice by a computer program which performs various routine calculations, such as background correction, specific radioactivity in each tissue sample, percentage of administered radioactivity in each organ, cross-over corrections of double isotopes, and statistical calculations of standard errors. The use of the computer program not only eliminated all our errors due to manual calculation, but most important of all, it reduced the data-processing time from a matter of several days to several hours. A similar study of the estimation of the blood volume in each organ of rats and rabbits will also be carried out in the future.

B. A Comparison of the Tissue Distribution of Liposomes Prepared by the Standard Method of Encapsulation and by the Method of Loading

The purpose of this study is to investigate the *in vivo* behavior of liposomes prepared by the new method of entrapping $^{111}\text{In}^{3+}$ ions in liposomes by using 8-HOQ ($^{111}\text{In}^{3+}$) described in 1A of this progress report. Ideally, the use of double isotopes labeling of liposomes will provide the most accurate information concerning whether or not liposomes prepared by the 8-HOQ ($^{111}\text{In}^{3+}$) loading method and liposomes prepared by the standard method of encapsulation by sonification have any subtle differences in terms of their blood clearance and distribution. This involves the loading of $^{111}\text{In}^{3+}$ to liposomes which have encapsulated a different radionuclide, such as $^{99\text{m}}\text{Tc}$, prior to the loading process.

The use of double tracers in one liposome can eliminate the variations in clearance rate and distribution from animal to animal. Attempts to encapsulate $^{99\text{m}}\text{TcO}_4^-$ in liposomes have not been successful due to the high permeability of liposome membrane to $^{99\text{m}}\text{TcO}_4^-$. The use of $^{99\text{m}}\text{Tc}$ -diethylenetriaminepentaacetic acid, DTPA ($^{99\text{m}}\text{Tc}$) as a marker has been tried. Work is in progress to eliminate the conversion of DTPA ($^{99\text{m}}\text{Tc}$) to $^{99\text{m}}\text{TcO}_4^-$ by oxidation inside the liposome.

Using DPPC: cholesterol (2:1 in molar ratio) liposomes entrapping ^{111}In by both methods, we have made a comparison of their blood clearance and tissue distribution in two sets of mice. The result is shown in Table 2, indicating that liposomes prepared by both methods of encapsulation are very similar. A manuscript describing the new method of loading radioactive metal ions to liposomes is in preparation.

C. Degradation of Liposomes in Liver

Since the method of loading a high specific radioactivity of ^{111}In to liposomes has only recently been developed. The data in the degradation of liposomes in tissues are very preliminary. So far we have only performed a few measurements on the physical states of liposomes in liver. Our result indicated that the degradation of liposome in liver appeared to continue at a rather significant rate even after the animal had been killed. Furthermore, the extent of degradation of liposomes in each organ was found to be different. Liposomes which are found in the fat and blood seem to be quite intact, whereas liposomes which are located in liver seem to be degraded most among all the organs.

II. Prolongation of Drug Carriers in Blood Circulation

1. A Comparison of the Distribution of Liposomes Administered by Intravenous and Intraperitoneal Injection

Previously, we have observed that DPPC: cholesterol (2:1 in molar ratio) unilamellar liposomes of about 650 Å in diameter stay in the blood circulation for several hours after intraperitoneal injection (3). The purpose of this study was to investigate whether or not the coating of a layer of peritoneal proteins on the surface of liposomes may increase their clearance time. A comparison of the liposomes administered by i.v. and i.p. injection will resolve this question.

During the early stage of calibrating the sizes of unilamellar liposomes as a function of the output power of the sonifer transmitted to the titanium microtip, we had a rather interesting and surprising observation. Liposomes prepared by sonificating at low output energy (about 45 watts) had a smaller diameter than liposomes obtained by sonificating at a higher output of energy (120 ~ 150 watts). From the study of the gel filtration chromatography in a Sepharose 4B column, liposomes prepared by sonificating at 45 watts had a diameter comparable to the smallest size liposomes of 350 Å in diameter (4), whereas liposomes obtained by sonificating at 120 ~ 150 watts had a diameter comparable to the size of 650 Å in diameter reported by us early (3). We believe the increase of the size of liposomes at 120 ~ 150 watts sonificating power might have been related to the excess foam or air bubbles during the sonificating at high output energy.

In our study, liposomes of both sizes were used for the comparison. The result of the preliminary study of small size liposomes (about 350 Å in diameter) is shown in Table 3. The result clearly indicated that the small size liposomes can stay in circulation for extended periods of time. The coating of a layer peritoneal proteins do not have any improvement in the clearance time of liposomes. However, the uptake of liposomes by liver appeared to be less, when liposomes were injected by intraperitoneal route. Furthermore, the uptake of liposomes by fat was quite significant, when liposomes were administered by intraperitoneal injection.

In the study of the distribution of medium size DPPC: cholesterol liposomes (Table 4), the uptake of liposomes by liver increased. A similar trend of a less hepatic uptake and more uptake by fat was also observed, when liposomes were given by intraperitoneal injection. Interestingly enough, the uptake of liposomes by the skin was very pronounced in the early periods after the intraperitoneal injection. This is quite in contrast to the distribution of small liposomes shown in Table 3. A similar observation of the uptake of unilamellar liposomes (about 650 Å in diameter) by skin has been reported in our early publication (3). This may suggest that the vessels and membranes of skin are responsible for liposome absorption, when liposomes are administered by intraperitoneal injection.

It is clear that from the above preliminary study, subtle changes in the tissue distribution of liposomes administered by different routes should be better addressed by using dual tracers.

2. The Characterization of Lipid-Protein Vesicles Made from Rabbit Serum

The initial goals of our study were to investigate whether or not lipid-protein vesicles can be made from rabbit serum, to determine the yield of encapsulation, and to explore the nature of such encapsulation. The rationale for pursuing this investigation was that although the report from the group of Kriss (5) has shown the radioactivity of $^{99m}\text{TcO}_4^-$ is associated with lipid-protein vesicles after gel filtration chromatography, there was no evidence to show that such association was a result of encapsulation alone. The association could conceivably come from the binding of $^{99m}\text{TcO}_4^-$ to some serum components which migrate accidentally with the liposome in the elution profile of a Sepharose 6B column. Furthermore, from our study we have found DPPC: cholesterol (2/1;M/M) liposomes are very permeable to $^{99m}\text{TcO}_4^-$. Thus, a close look of the system of lipid-protein vesicles will be useful.

Five different markers have been used to investigate this problem. These markers are $^{99m}\text{TcO}_4^-$, 0.106M sodium phosphate at PH7.4, EDTA ($^{111}\text{In}^{3+}$), EDTA (^{99m}Tc), and DTPA (^{99m}Tc). The results of the preliminary study can be summarized as follows:

- (i) When $^{99m}\text{TcO}_4^-$ was cosonificated with serum, about 0.24% of the total radioactivity was associated with the fractions which could contain lipid-protein vesicles. On the other hand, only 0.036% of the total radioactivity was associated with fractions of lipid-protein vesicles, when $^{99m}\text{TcO}_4^-$ was added after the sonification of serum. This may suggest that the 7 folds increase of radioactivity in the fraction of lipid-protein vesicles could be due to encapsulation. However, there is still lack of a solid proof of encapsulation. The study of the leakage of material from the fractions of lipid-protein vesicles could conceivably lend some support on this.
- (ii) In the experiment of encapsulation, where EDTA ($^{111}\text{In}^{3+}$) was cosonificated with serum, about 2% of the total radioactivity was associated with the fractions of lipid-protein vesicles. In the control, only 0.35% of radioactivity was associated with the fractions of lipid-protein vesicles, when EDTA ($^{111}\text{In}^{3+}$) was incubated with serum which had been sonicated prior to the addition of EDTA ($^{111}\text{In}^{3+}$). The study of the leakage of EDTA ($^{111}\text{In}^{3+}$) from the fraction of lipid-protein vesicles by the technique of dialysis indicated that about 33% of the radioactivity in the fraction of lipid-protein vesicles can be dialyzed out over a period of 12 hours in both experiments of encapsulation and control. However, in the presence of 1% Triton X-100, the release of EDTA ($^{111}\text{In}^{3+}$) from the fractions of lipid-protein vesicles was 60% for the experiment of encapsulation and 40% for the control. Work is in progress to investigate the physical states (bound or free state) of EDTA ($^{111}\text{In}^{3+}$) in the fraction of lipid protein vesicles by the technique of perturbed angular correlation of gamma radiation.

- (iii) The encapsulation of two other markers of ^{99m}Tc , namely EDTA (^{99m}Tc) and DTPA (^{99m}Tc) by serum lipid turned out to be not very successful because of the extensive binding of these two markers with serum proteins. Work is in progress to use sodium phosphate as a different marker.

From the above preliminary results, the encapsulation of materials by serum lipids seemed to be feasible. However, from the measurement of the concentration of serum lipid, our estimation was only about 2 mg serum phospholipids per one ml of rabbit serum. We have been able to increase the concentration of serum phospholipids 5 ~6 folds by using a Millipore ultrafiltration filter system. Work is in progress to investigate whether or not the encapsulation of a marker with lipid-protein vesicles will increase, when the concentrated serum is used.

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Table 1. Distribution of Erythrocytes Labeled by $^{111}\text{In}^*$

Organs	% injected dose per organ
Liver	3.77 ± 1.18
Kidneys	0.95 ± 0.15
Spleen	0.96 ± 0.46
Heart	0.76 ± 0.30
Lung	2.25 ± 1.20
Intestines	1.11 ± 0.15
Fat	0.54 ± 0.24
Skin	4.65 ± 3.56
Muscles and Bones	15.96 ± 3.56

* The blood volume of BALB/c mouse of about 25 g was estimated to be 8.96% of the body weight.

Table 2. Comparison of the distribution of small size DPPC: cholesterol unilamellar liposomes prepared by 8-HOQ ($^{111}\text{In}^{3+}$) loading procedure and by standard method of encapsulation by sonification * †

	Liposomes prepared by 8-HOQ($^{111}\text{In}^{3+}$) loading procedure				Liposomes prepared by encapsulation procedure		
	15 min.	30 min.	1 hour	3 hours	5 min.	1 hour	3 hours
Blood	64	57	53	38	71	48	32
Liver	4	5	4	8	3	8	16
Kidneys	3	3	6	7	2	7	6
Spleen	0	0	0	1	0	1	1
Heart	1	0	1	0	0	1	0
Lung	0	1	1	0	1	0	0
Intestines	2	4	4	7	2	4	3
Fat	2	1	1	2	0	1	2
Skin	5	7	7	13	3	6	12
Muscles and Bones	16	19	21	22	16	23	24

* All the numbers are expressed as the percentage of dose per organ after the blood background correction.

† The administration of liposomes was by intravenous injection.

Table 3. Distribution of small size DPPC: cholesterol unilamellar liposomes *

	<u>Intravenous injection</u>					<u>Intraperitoneal injection</u>				
	5 min.	1 hour	3 hours	5 hours	23 hours	1 hour	3 hours	5 hours		
Blood	71	48	32	26	5	39	30	27		
Liver	3	8	16	20	29	8	11	14		
Kidneys	2	7	6	5	7	3	3	2		
Spleen	0	1	1	1	2	0	1	1		
Heart	0	1	0	0	0	0	0	0		
Lung	1	0	0	0	0	0	1	0		
Intestines	2	4	3	6	9	5	7	11		
Fat	0	1	2	2	3	12	11	13		
Skin	3	6	12	11	14	8	12	11		
Muscles and bones	16	23	24	24	27	22	20	16		

* All the numbers are expressed as the percentage of dose per organ after the blood-background correction.

Table 4. Distribution of medium size DPPC: cholesterol unilamellar liposomes *

	<u>Intravenous injection</u>				<u>Intraperitoneal injection</u>			
	45 min.	2 hours	4 hours	66 hours	10 min.	50 min.	2 hours	4 hours
Blood	64	30	25	1	3	19	23	13
Liver	17	43	40	49	6	21	32	36
Kidneys	2	2	2	4	1	2	4	2
Spleen	1	3	6	6	0	2	6	5
Heart	0	0	0	0	0	0	0	0
Lung	0	0	0	0	0	0	0	0
Intestines	2	3	5	5	8	8	9	14
Fat	0	1	1	0	5	6	4	3
Skin	5	5	7	11	39	21	14	9
Muscles and bones	10	11	12	17	30	20	9	17

* All the numbers are expressed as the percentage dose per organ after the blood-background correction

Glossary

DPPC: L- α -dipalmitoyl phosphatidylcholine

8-HOQ: 8-hydroxyquinoline

8-HOQ($^{111}\text{In}^{3+}$): the complex of 8-HOQ with $^{111}\text{In}^{3+}$.

DTPA: diethylenetriaminepentaacetic acid

DTPA($^{111}\text{In}^{3+}$): the complex of DTPA with $^{111}\text{In}^{3+}$

DTPA($^{99\text{m}}\text{Tc}$): the complex of DTPA with $^{99\text{m}}\text{Tc}$ ions

PAC: perturbed angular correlation

PBS: phosphate buffer saline

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